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DIGESTION AND DISTRIBUTION OF TRIPALMITOYLGLYCEROL IN *DIPLODON DELODONTUS* (MOLLUSCA, BIVALVIA)

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Abstract

Fat digestion, absorption, and transport in the fresh water mollusc *Diplodon delodontus* were studied after 1 and 6 h of force-feeding ^{14}C tripalmitoylglycerol and 1-^{14}C palmitic acid. In a 1 h period, the mollusc was able to hydrolyze more than 50% of the triacylglycerol to free acids monoacyl and diacylglycerols. Digestion and absorption was completed before the 6 h feeding, and most of the label was eliminated. Hydrolysis occurred primarily in the stomach. The mollusc absorbed the products of hydrolysis and apparently triacylglycerol molecules, too. During the period of tripalmitoylglycerol digestion (1 h), the labeled palmitic acid was transported by the hemolymph in the free acid, monoacylglycerol, and triacylglycerol fractions that were also the principal components found in the stomach. During the post absorption period (6 h) the label was principally bound to triacylglycerols. When 1-^{14}C palmitic acid was fed to *D. delodontus,* the acid was absorbed and the label transported exclusively in the free acid fraction of the hemolymph during the first hour. At 6 h ~75% was still transported as free acid and the rest as triacylglycerol. The free palmitic acid was incorporated in the soft tissues of the mollusc and slowly esterified to triacylglycerols.

Introduction

Since Yonge's (1926) work, several reports have been published regarding lipid digestion in bivalve molluscs. These are generally based on histological and histochemical methods. The original controversy between intracellular or extracellular digestion of fats apparently ended when George (1952) found evidence employing histochemical techniques that extracellular hydrolysis of triacylglycerol took place in the stomach of lamellibranchia molluscs. Some authors suggested that lipolytic enzymes might be localized in vesicles excreted by digestive glands (Mansour and Zari, 1946; Morton, 1956) while others also detected lipase activity in the crystalline style (Hozumi, 1959; 1961; Payne, 1978; Palmer, 1979). Patton and Quinn (1973) reported a quantitative study on lipid digestion in a marine clam. Using homogenates of the crystalline style of *Spisula solidissima,* they investigated optimal conditions for lipase activity and showed that it catalyzed the hydrolysis of a wide variety of substrates but had low activity for triacylglycerols.

This information indicates that the digestion of lipids is not yet fully understood in bivalve molluscs and that little is known about the absorption and distribution of dietary lipids. To trace lipids, the fresh water mollusc *Diplodon delodontus* was force-

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fed ^{14}C tripalmitoylglycerol and 1-^{14}C palmitate. Tripalmitoylglycerol was chosen since Pollero *et al.* (1983) and Irazú *et al.* (1984) have shown that palmitoyl glycerides are the predominant lipids in the natural diet of the mollusc. Free acid was also administered to investigate triacylglycerol resynthesis from the acid absorbed in the digestive tract.

Materials and Methods

Materials

Adult *Diplodon delodontus* (10–12 cm) were collected from a pond close to the La Plata River (34°53′ S, 57°50′ W) Berisso, Buenos Aires Province, Argentina at 1.5 m depth. Specimens were maintained at room temperature in aquaria with aerated water and pond sediment for a week before the experiments were performed.

Tripalmitoylglycerol (^{14}C) (51.5 mCi/mmol) labeled in carbon 1 of palmitic acid and 1-^{14}C palmitic acid (58 mCi/mmol) (Amersham International Ltd., England) were used as radioactive tracers. Non-radioactive tripalmitoylglycerol and palmitic acid (Nu-Check Prep, U.S.A.) were used to dilute labeled materials for the experiments.

Feeding with labeled substrates

V-shaped holes were cut in the shells of the molluscs with a hand saw to expose the mouth. Animals survived this treatment for periods up to 24 h. Each clam was fed, with a water suspension of 6.25 μCi (3.1 μmol) tripalmitoylglycerol and soy bean powder (52 mg), through a canula located in the mouth. Two groups of four animals each were maintained in these conditions for one and six hours, respectively. After that time the shell valves were opened, cutting the aductor muscles with a bistoury, and the hemolymph was immediately obtained by cardiac puncture. Approximately 1 ml of hemolymph was recovered per animal. The stomach and its content, intestine with its content, and digestive diverticula were carefully dissected. All other soft tissues were assembled in one pool. Two other groups of four clams each were also fed with ammonium palmitate (3 μCi/0.5 μmol each clam) for one and six hours. The procedures followed for the substrate administration and dissection of tissues were similar to those described for the tripalmitoylglycerol assays.

Lipid extraction and analysis

Dissected organs and the remaining soft tissues were homogenized in a Tri-R homogenizer with chloroform-methanol (2:1 v/v) at the rate of 20 ml per gram of tissue and total lipids extracted by the procedure of Folch *et al.* (1957). Radioactivity was measured in aliquots of the extracts dissolved in Bray's scintillation solution. Total lipids were analyzed by thin-layer chromatography on plates of Silicagel G plus 10% boric acid, using hexane-diethyl ether-acetic acid (60:40:1.5 v/v) as development solvents. Lipids were identified on the plates by comparing the R_f with the corresponding standards. The radioactivity distribution among the lipids was measured on the plates with a scanner counter apparatus (Berthold, Germany). Peak areas were calculated by triangulation.

Results

Tripalmitoylglycerol

Table I shows the distribution of radioactivity found in different organs of the clam after force-feeding ^{14}C tripalmitoylglycerol for one and six hours. After the first

TABLE I

Radioactivity distribution in Diplodon delodontus *tissues fed* ^{14}C *tripalmitoylglycerol*

Tissues	Total radioactivity (d.p.m. × 10^3)		Lipid radioactivity (d.p.m. × 10^3/mg of lipid)		Radioactivity recovered in the lipids (percent)[1]	
	1 h	6 h	1 h	6 h	1 h	6 h
Stomach	32,333.4	100.5	1530.9	28.7	53.9	0.2
Intestine	4748.7	95.0	949.7	25.0	7.9	0.2
Digestive diverticula	1232.7	2197.0	94.8	22.5	2.1	3.5
Hemolymph	4435.7	72.0	252.0	51.4	7.4	0.1
Other soft tissues	3386.3	3932.0	6.9	8.5	5.6	6.4

Total radioactivity administered per pool was 55,000 × 10^3 d.p.m.
Results were obtained from pools of four animals.
[1] Percent of administered radioactivity.

hour of force-feeding the labeled lipid, approximately half of the radioactivity administered still remained in the stomach. Only ~13% was recovered in tissues not belonging to the digestive tube. After 6 h only 10% of the administered radioactivity was recovered and only a small percentage (0.2%) remained in the stomach. Soft tissue lipids collected the highest amount. Therefore, absorption of the lipid was already complete at 6 h and a large part of the palmitic acid had been consumed or eliminated with the feces.

To investigate the fate of the labeled tripalmitoylglycerol, the radioactivity distribution in the lipids of each tissue separated by thin-layer chromatography was determined (Table II). In the stomach, at the first hour less than 50% of the labeled palmitate of tripalmitoylglycerol appeared in the triglyceride fraction and the rest in the products of hydrolysis, principally free palmitic acid and less proportions in monoacylglycerols and diacylglycerols. These results indicate that lipase digestion of

TABLE II

Percent distribution of radioactivity in the lipids of Diplodon delodontus *after 1 and 6 h feeding* ^{14}C *tripalmitoylglycerol*

Tissue	Period (h)	Free fatty acids	Monoacyl-glycerols	Diacyl-glycerols	Triacyl-glycerols
Stomach	1	40.0	11.4	5.7	42.9
	6	22.6	2.0	—	75.4
Intestine	1	9.3	6.2	6.7	77.8
	6	4.4	4.1	—	91.5
Digestive diverticula	1	7.3	6.1	3.9	82.7
	6	9.0	7.1	—	83.9
Hemolymph	1	44.8	27.2	—	28.0
	6	4.4	5.5	—	90.1
Other soft tissues	1	7.5	—	—	92.5
	6	5.3	8.4	4.2	82.0

No label was found in phospholipids.
Results were obtained from pools of four animals.

the tripalmitoylglycerol occurred in the stomach during the first hour. The same products of hydrolysis were found in the intestine and digestive diverticula but in lesser amounts and percentages.

After 6 h the small amount of radioactivity in the stomach mainly belonged to the triacylglycerol fraction and only a low percentage was recovered in the free acids and monoacylglycerols (Table II). A similar distribution with even less labeling in products of hydrolysis was found in intestine and digestive diverticula.

The lipids of hemolymph and other soft tissues were also labeled in the first hour of the experiment. Except for hemolymph in which most of the label was found in fractions with R_f corresponding to monoacylglycerols and free fatty acids, more than 90% of the radioactivity in the other tissues was recovered in the triacylglycerols. Therefore, in the first hour after food intake, part of the triacylglycerol fed had been digested, the products absorbed, and distributed in the different organs.

After 6 h few radioactive lipids remained in hemolymph (Table I) and these consisted principally of triacylglycerols (Table II). In the other tissues most of the radioactivity was also found in triacylglycerols.

Palmitic acid

The above experiments gave some information about digestion and absorption of a triacylglycerol by the mollusc. However, they did not provide definitive information about the resynthesis of triacylglycerols in the digestive tube tissues from free fatty acids. For this reason, molluscs were force-fed the ammonium salt of 1-^{14}C palmitic acid. Table III shows that after the first hour of administration all radioactivity found in the digestive tube, organs, and hemolymph remained unaltered as free acid. A similar behavior has been reported for longer time periods in *Crassostrea gigas* (Allen and Conley, 1982). However, in other organs a small part of the label was also attached to triacyl and diacylglycerols. Therefore, no esterification of the absorbed acid occurred under these conditions in the digestive tube cells and the acid was transported as such by the hemolymph. Only target tissues would esterify part of the acid to synthesize neutral lipids.

TABLE III

Percent distribution of radioactivity in the lipids of Diplodon delodontus *after 1 and 6 h-feeding 1-^{14}C palmitic acid*

Tissue	Period (h)	Free fatty acids	Monoacyl-glycerol	Diacyl-glycerol	Triacyl-glycerol
Stomach	1	100	—	—	—
	6	74.7	—	7.1	18.2
Intestine	1	98.4	—	—	1.6
	6	83.7	—	2.0	14.3
Digestive diverticula	1	100	—	t	t
	6	61.7	—	9.9	28.4
Hemolymph	1	100	—	—	t
	6	76.5	—	—	23.5
Other soft tissues	1	90.3	t	4.7	5.0
	6	71.9	0.6	7.6	19.9

No label was found in the phospholipids.
Results were obtained from pools of four animals.

After 6 h of 1-^{14}C palmitate ingestion, the incorporation of the label in triacylglycerols and diacylglycerols in the digestive tract organs was detected (Table III). But even at this time most of the palmitic acid remained unesterified. The hemolymph still transported the palmitic acid predominantly in the free form but 23.5% was now detected in the triacylglycerols. In the other organs, most of the label was still detected in the free acid but the fraction esterified to diacyl- and triacylglycerols had increased.

Discussion

After force-feeding ^{14}C tripalmitoylglycerol, the radioactivity was found in diacylglycerol, monoacylglycerol, and free fatty acids of the stomach (Table II). Consequently, the presence of these lipids demonstrates the existence of hydrolytic activity in this part of the digestive tube and confirms earlier experiments done with other molluscs (Hozumi, 1959; Patton and Quinn, 1973; Payne, 1978; Palmer, 1979). Non-enzymatic hydrolysis was eliminated since the incubation of 1-^{14}C tripalmitoylglycerol with deoxycholate and $CaCl_2$ in phosphate buffer pH 7.1 for 1 h at 25°C did not produce labeled free acids. In intestine and digestive diverticula the proportion of hydrolysis products of the triacylglycerol is remarkably lower suggesting that the largest part of the tripalmitoylglycerol lipolysis took place in the stomach. However, this experiment does not eliminate the possibility of lipase activity in intestine and diverticula produced by enzymes arriving from the stomach or even produced in these tissues.

The present data do not establish clearly in which part of the digestive tube dietary lipids are absorbed. However, the high lipase activity of the stomach in addition to the abundant villi and rich vascularization in the stomach wall (Huca *et al.*, 1982) suggest that most lipid absorption takes place at this level.

Tables I to III indicate that absorbed lipids are distributed by the hemolymph to the different organs. Tables II and III indicate that hemolymph reflects approximately the lipid composition of the stomach. After the first hour of feeding, the tripalmitoylglycerol, triacylglycerol, diacylglycerol, monoacylglycerol, and free fatty acids of the gastric tube were labeled except for the diacylglycerols; the same lipids in a rather similar proportion were found in the hemolymph.

When the free fatty acid was administered (Table III) the radioactivity was detected only in the free acid fraction of the hemolymph. Therefore, these data indicate that the digestive tube walls absorb free palmitic acid (Tables II, III) and monopalmitoylglycerol (Table II), and they can also be transported without transformation by the hemolymph. Therefore, the mechanism of fatty acid transport in mollusc hemolymph would differ significantly from mammals that carry ingested fatty acids in triacylglycerol molecules incorporated to chylomicrons. They would even differ from more related animals, *e.g.*, insects that carry them as diacylglycerols in specific lipoproteins (Fichera and Brenner, 1982).

However, labeled triacylglycerols were found in *D. delodontus* hemolymph after 1-^{14}C tripalmitoylglycerol was administered in the food (Table II). This triacylglycerol present in the hemolymph could be of two origins. It could be produced by resynthesis in the mucous cells of the digestive tube wall from the labeled products derived from the hydrolysis of administered ^{14}C tripalmitoylglycerol. This reaction takes place in mammals in which monoacylglycerol and diacylglycerols may be converted to triacylglycerols by acylation in the intestine wall. Absorbed free acids are also converted to acyl-CoA and then esterified to glycerol phosphate produced in glycolysis or monoacylglycerols and transformed at the end into triacylglycerols (Johnston, 1963). Alternatively, one should consider the direct absorption of tripalmitoylglycerol. When 1-^{14}C palmitic acid was administered to the mollusc for 1 h,

the label was found in the free acid and not in the triacylglycerols of the hemolymph (Table III), suggesting little esterification of tagged free palmitic acid to triacylglycerols in the digestive tube cells. In view of these results, the presence of labeled triacylglycerols in the hemolymph of the mollusc after 1 h of force-feeding 1-^{14}C tripalmitoylglycerol (Table II) would suggest the second alternative which considers that part of triacylglycerol molecules could be directly absorbed through the digestive walls by the *Diplodon delodontus.*

However, in the 6-h experiment, administered 1-^{14}C palmitic acid also labeled a triacylglycerol fraction of hemolymph, stomach, intestine, and diverticula. Therefore, the incorporation of the free acid into triacylglycerols would be produced at longer periods, but we can not yet establish which could be the responsible tissue for the esterification.

The increase of time in the experiment also increased the labeling of triacylglycerols of other soft tissues indicating very probably a local biosynthesis from the free acid provided by the hemolymph, since labeled diacylglycerols were also found in these tissues but not in hemolymph (Table III). This triacylglycerol biosynthesis is produced slowly since after 6 h about 62% or more of the label still remained as free palmitic acid.

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